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Identification of Biomarkers Associated with the Healing of Chronic Wounds

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CONTRACTING ORGANIZATION:

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14. ABSTRACT It is the objective of this study to identify the biochemical differences in the burn fluid of burns with hypertrophic scarring and those without. The findings of this study are intended to facilitate the development of diagnostic tools, which could be used to evaluate the healing process and develop therapeutic treatments. A porcine burn model has been used to evaluate healing. PIXIES was used to analyze the cytokines and growth factors present in the burn wounds.					
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INTRODUCTION

Burns are among the most common injuries in modern conflicts. In recent years burn patient mortality has significantly decreased due to early wound excision and more immediate skin grafting. With increased survivorship comes the risk of hypertrophic scarring, a common complication associated with healed deep burns. Hypertrophic scarring often results in serious psychological and physical effects. The pathophysiology of hypertrophic scar formation is not clear and it is not understood why some patients develop hypertrophic scarring while others do not. Because hypertrophic scarring is the result of a protein imbalance at the injury site, the biochemical characterization of the burn wound from the time of injury until closure in parallel with the identification of the differences in burn fluid and burn patient sera is the important first step in developing a treatment to prevent hypertrophic scarring.

This proposal will assess the biochemical profiles of healing burns and compare the profiles from burns with hypertrophic scarring versus those without, thereby testing the hypothesis that specific quantifiable biochemical differences in the sera and burn fluid exist between burn patients that develop hypertrophic scarring and those that do not.

BODY

STATEMENT OF WORK

Continuation

Burn Fluid and Patient Sera Biochemical Analysis as an Indicator of Aberrant Wound Repair and Hypertrophic Scarring

Phase I:

Technical Objective 1: Characterize the protein biochemistry of burn wounds.

- a. Analyze wound fluid samples to determine proteins present
- a. Identify trends present in burns as healing occurs

Technical Objective 2: Characterize the protein biochemistry in the sera of subjects with burn wounds.

- a. Analyze sera to determine the proteins present
- b. Identify trends present in subjects with burns during healing

Technical Objective 3: Assess the presence of hypertrophic scarring.

- a. Burn Scar Index (Vancouver Scar Scale) parameters of scar will be assessed

- b. Identify subjects with hypertrophic scarring burn wounds

Technical Objective 4: Correlate the differences between the sera and burn fluid samples during healing and identify biochemical differences between hypertrophic scarring and non-hypertrophic scarring subjects.

- a. Correlate the trends in wound and sera biochemistry during healing
- b. Correlate clinical outcome with biochemistry
- c. Identify the differences present in sera and wound exudates in samples from subjects with hypertrophic scarring

Phase II:

Technical Objective 1: Develop a porcine model for burn wounds (second degree - superficial and deep).

- a. Develop methods to reproducibly induce cutaneous thermal injuries in porcine tissue model.
- b. Collect wound fluid from thermally injured swine for proteins of clinical interest, based upon those identified in Phase I of this project.

Technical Objective 2: Characterize the protein biochemistry of porcine wound fluids.

- a. Analyze burn wound fluid by both ELISA and PIXIES.
- b. Compare results from PIXIES with those from ELISA.

Technical Objective 3: Evaluate and validate porcine data with those obtained from Phase I studies.

- a. Compare wound fluid biochemistry from thermally injured swine to that from normally-healing human wound fluid from Phase I of the study.

Phase I

Technical Objectives 1, 2, & 3:

Completion of all Phase I technical objectives has been delayed due to slow enrollment. Dr Chung, Medical Director, Burn ICU Task Area Manager, Clinical Trials in Burns and Trauma US Army Institute of Surgical Research and Dr Edsberg are working towards enrollment at the USAISR for the study.

Dr. Edsberg is working with Cathy Rauschendorfer, RN, Clinical investigator Coordinator at the USAISR to finalize IRB approval for the protocol and begin enrollment. The review was completed and the revised protocol has been submitted to the IRB in Tx for approval (9/15/14).

The final protocol will be submitted to the Daemen College IRB, as well as to the USAMRMC, ORP, and HRPO prior to any enrollment. A one-year no cost extension has been granted for the project.

Phase II

Technical Objective 1: Develop a porcine model for burn wounds (second degree – superficial and deep)

- a. *Develop methods to reproducibly induce cutaneous injuries in porcine tissue model*
- b. *Collect wound fluid from thermally injured swine for proteins of clinical interest, based on those identified with phase I of this project*

Completed

Technical Objective 2: Characterize the protein biochemistry of porcine wound fluids

- a. *Analyze burn wound fluid by both ELISA and PIXIES*
- b. *Compare results from PIXIES with those from ELISA*

a. PIXIES and ELISA analysis were performed on porcine wound fluids for the following proteins: KGF, IL-1, IL-6, IL-8, IL-12, TNF-alpha. In addition, the PIXIES platform has been used for TGF-alpha and beta, VEGF and EGF detection in untreated biological samples. Spiked biological samples were also assessed to establish correlations. Only KGF, IL-1, IL-6, IL-8 and IL-12 were found in the biological specimens.

- b. *Compare results from PIXIES with those from ELISA*

Completed

Technical Objective 3: Evaluate and validate porcine data with those obtained from Phase I studies

Compare wound fluid biochemistry from thermally injured swine to that of normally-healing human wound fluid from Phase I of the study

Porcine wound fluids await additional analyses, which are part of Phase I. Additional analysis will be run with samples from human subjects when enrollment has increased.

Key Research Accomplishments

- Development of porcine thermal injury model system (Phase II)
- Collection of porcine burn wound fluid at several time intervals post-injury (Phase II)
- Analyses of wound fluids for proteins using PIXIES (Phase II)
- Demonstrated viability of PIXIES platform for assessing wound fluid biochemistry (Phase II)

Reportable Outcomes

- Collaboration with Dr. Chung has led to additional research collaborations in Texas in the area of burn research.

Several related peer-reviewed journal publications have derived from DOD sponsorship:

- Z. Tao, E.C. Tehan, R.M. Bukowski, Y. Tang, E.L. Shugart, W.G. Holthoff, A.N. Cartwright, A.H. Titus and F.V. Bright, "Templated Xerogels as Platforms for Biomolecule-less Biomolecule Sensors," *Anal. Chim. Acta* 2006, 564, 59-65.
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- P.J.R. Roche, M.C-K Cheung, K.Y. Yung, A.G. Kirk, V.P. Chodavarpu and F.V. Bright, "Application of Gold Quenching of Luminescence to Improve Oxygen Sensing using a Ruthenium (4,7-diphenyl-1,10-phenanthroline)₃ Cl₂:TEOS Thin Film," *Sens. Actu.: B Chem.* 2010, 147, 581-586.
- S.A. Burns, R. Hard, W.L. Hicks Jr., F.V. Bright, D. Cohan, L. Sigurdson and J.A. Gardella Jr., "Determining the Protein Drug Release Characteristics and Cell Adhesion to a PLLA or PLGA Biodegradable Polymer Membrane," *J. Biomed. Mater. Res. Part A* 2010, 94A, 27-37. L. Yao, K.Y. Yung, R. Khan, V.P. Chodavarapu, and F.V. Bright, "CMOS Imaging of Pin-Printed Xerogel based Luminescent Sensor Microarrays," *IEEE Sens. J.* 2010, 10, 1824-1832.
- E.L. Holthoff and F.V. Bright, "Photophysics Associated with Site Selectively Templated and Tagged Xerogel Sensor Platforms," *Appl. Spectrosc.* 2010, 64, 714-719.
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- D.S. Daivasagaya; L. Yao, K.Y. Yung, M. Hajj-Hassan, M.C. Cheung, V.P. Chodavarapu, * F.V. Bright, "Contact CMOS Imaging of Gaseous Oxygen Sensor Array," *Sens. Actuat. B: Chem.* **2011**, 157, 408-416.
- K.Y. Yung, H. Xu, K. Liu, G.J. Martinez, F.V. Bright*, M.R. Detty and A.N. Cartwright, "Hybrid Oxygen-Responsive Reflective Bragg Grating Platforms," *Anal. Chem.* **2012**, 84, 1402-1407.
- M.C. Chung, K.Y. Yung, H. Xu, N.D. Kraut, K. Liu, V.P. Chodavarapu, A.N. Cartwright and F.V. Bright*, "Porous Nanostructured Encapsulation and Immobilization Materials for Optical Biosensors," *J. Selec. Top. Quant. Electron.* **2012**, 18, 1147-1159.
- N.D. Kraut, J.D. Brattlie, R.E. Deuro, M.M. McGoorty and F.V. Bright*, "High-Throughput Screening System for Creating and Assessing Surface-Modified Porous Silicon," *Appl. Spectrosc.* **2012**, 66, 1171-1178.
- Z. Zhan, B. Zhou, Z. Fu, F.V. Bright, A.N. Cartwright and A.H. Titus*, "Filterless Optical Oxygen Sensor Based on a CMOS Buried Double Junction Photodiode," *Sens. Actu : Chem. B* **2013**, 176, 729-735.

Conclusion

- Phase I delayed due to slow subject enrollment
- All but one of Phase II objectives have been met.
- Technical objective 3 of Phase II is delayed by the enrollment issues in Phase I.